LETHAL AND SEMILETHAL MUTATION FREQUENCY OF THE FIRST AND SECOND CHROMOSOMES IN TWO NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER*

A Thesis

Presented to

The School of Graduate Studies

Drake University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

---

by

Frederick M. Krick

August 1986

LETHAL AND SEMILETHAL MUTATION FREQUENCY OF THE FIRST AND SECOND CHROMOSOMES IN TWO NATURAL POPULATIONS OF DROSOPHILA MELANOGASTER

by

Frederick M. Krick

Approved by Committee

Michael E. Myszewski
Dr. Michael E. Myszewski

Dean A. Hoganson
Dr. Dean A. Hoganson

W Scott Wood
Dr. W. Scott Wood

W. Russell Abell
Dr. W. Russell Abell
Dean of the School of Graduate Studies

LETHAL AND SEMILETHAL MUTATION FREQUENCY OF THE FIRST AND SECOND CHROMOSOMES IN TWO NATURAL POPULATIONS OF DROSOPHILA MELANOGASTER

An abstract of a Thesis by
Frederick M. Krick
August 1986
Drake University

The problem. The present study was undertaken to examine potential genetic diversity as measured by the lethal gene frequencies between two natural populations of Drosophila melanogaster.

High levels of trichloroethylene (TCE) and other suspected mutagens and carcinogens, i.e., vinyl chloride, chloroform, etc., have reportedly been found in subsurface soil and waters near downtown Des Moines, Iowa.

Lethal gene frequency of the first (X) and second chromosomes of the toxic population sample (captured within the toxic plume locale), was determined by genetic analysis and compared to that of a "control" population sample captured in an area believed to be free of chemical contamination.

Procedure. The Basc technique for determining X-linked lethal mutation frequency and the Cy/Pm method for determining second chromosome lethal mutation frequency were employed. Mutation frequencies were calculated and data was analyzed by the Chi-square method.

Findings. The X-linked lethal mutation frequency of the control population sample was found to be 0%. Compared with the toxic population X-linked lethal mutation frequency of 1.47%, the difference is not significant.

Drastic (lethal + semilethal) mutation frequency of the control population second chromosomes was found to be 48%. When compared to the toxic population second chromosome drastic mutation frequency of 56%, the difference is not significant.

Conclusion. The data obtained from this study showed that the chemical contamination of the toxic population sample habitat had no significant effect on the frequency of X-linked or second chromosome lethal mutation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION AND REVIEW OF THE LITERATURE

Modern mutation theory is based largely on the Watson and Crick (1953) model for DNA structure and replication. Although much investigation is currently focused upon the molecular aspects of mutagenesis, classical mutation studies utilizing Drosophila provide a valuable source of information pertinent to evolution. It is not surprising, therefore, that many investigators have sought to understand the nature, induction, and effects of mutation in Drosophila based studies.

Theoretically, the number of mutations resulting in lethal changes should greatly outnumber those producing visible, detectable changes (Muller, 1928). "A lethal may represent a change within a single locus, a gene, or it may be a structural change in a chromosome such as a deficiency for one or several adjacent genes" (Dobzhansky and Wright 1941, p. 48). Muller (1928) realized that a quantitative study of mutation, using the frequency of origin of lethals as an index of mutation rate was possible. Another benefit in utilizing lethals lies in the objective detection of such mutations with the elimination of a "personal equation" often necessary in classification of phenotypic variants (Muller, 1928).

Since Muller's (1927) discovery that ionizing radiations (X-rays) induce mutation, many investigators

have sought to understand the relation between extrinsic variables producing mutations and the physical ramifications so induced. Naturally the frequency and rate of mutation induced by radiation (Lefevre, 1981; Muller, 1927; Muller et al., 1954; Spencer and Stern, 1948; Wallace, 1950), various temperatures (Dobzhansky and Spassky, 1944; Ives, 1943; Muller, 1928; Tobari, 1966), environmental differences related to geographic sampling of wild populations (Band, 1964; Band and Ives, 1963; Dobzhansky, 1939; Dobzhansky and Queal, 1938; Dobzhansky and Spassky, 1953; Demerec, 1937; Dubinin, 1946; Ives, 1945) and various chemicals with suspected mutagenic or carcinogenic action (Ames et al., 1975; Herskowitz, 1951; Lee et al., 1983; Nagao and Sugimura, 1978) have been examined.

Muller (1928) was instrumental in the early development of techniques for obtaining gene mutation frequency data utilizing Drosophila melanogaster. Drosophila has been the organism of choice for many experimental biologists for a number of reasons: low maintenance costs, small size, short generation (9-11 days), and readily available defined genetic test systems.

The "C_1" factor (discovered by Sturtevant), an inversion which suppresses crossing-over, as well as the incorporation of sterility factors in balanced chromosomes, greatly facilitated the expansion of Drosophila test systems (Muller, 1928). Methods for determining sex-linked

(Muller, 1928; Muller and Altenburg, 1919) and autosomal (Muller, 1928) lethal mutation rates in Drosophila are also notable early advances.

A large amount of data has accumulated on the relationship between radiation and induced gene mutations. The first experimental induction of increased lethal (both dominant and recessive) and semilethal gene mutation frequencies in D. melanogaster was reported by Muller (1927), following X-ray treatment of sperm, oöcytes, and oögonial cells. This was coincident with the experimental induction of somatic mutations in barley following X-ray and radium treatment of germinating seeds (Stadler, 1928).

Early radiation geneticists suggested the r-dose/ mutation frequency relation to be linear (Spencer and Stern, 1948), however later investigation found a marked flattening of the lethal frequency-dosage curve at high doses (Muller et al., 1954). It was suggested that this flattening was a result of induced chromosomal breakage within more susceptible germ cells. Albeit mutations were occurring in response to the radiation, chromosomal breakage resulted in diminished survival and detection.

Altenburg, working in 1918 and 1919, was the first to demonstrate the dependence of sex-linked lethal mutation rate on temperature (Altenburg, 1919, unpublished paper: reported in Muller, 1928). Although this dependence has been confirmed for sex-linked

chromosomes in Drosophila (Muller and Altenburg, 1919; Muller, 1928), results for autosomes have varied. Ives (1943, 1945), studying ten second chromosome lines of Florida D. melanogaster found no significant effect on lethal mutation rate following temperature shock. Heterogeneity of genetic factors influencing lethal mutation was suggested. An analysis of second and fourth chromosomes of D. pseudoobscura resulted in evidence that viability of homozygotes with respect to temperature was indeed variable (Dobzhansky and Spassky, 1944). Here the same genotype was noted to be lethal at 25.5°C, semilethal at 21°C, and nearly normally viable at 16.5°C. Alternately, others (Band and Ives, 1963; Tobari, 1966) have found significant temperature-lethal mutation dependence for autosomes.

The lethal mutation frequency in natural (wild) Drosophila populations is mediated by many variables related to the geographic placement of the population: seasonal cycle, rainfall, and temperature trends.

Olenov et al. (1939) was the first to suggest a relationship between the seasonal cycle and lethal frequency (reported in Dubinin, 1946). With the advance of the breeding season, subsequent numerical expansion of populations is accompanied by increased lethal gene saturation. Increasing lethal gene homozygosity, due to inbreeding, results in lethal frequency decline.

Dubinin (1946) suggests this as evidence for rapid evolutionary change within populations; this change being governed by cyclic seasonal change.

A study of combined weather variables (Band and Ives, 1968), found a significant positive correlation between summer rainfall and drastic (lethal + semilethal) gene frequency. It was suggested that rainfall is positively correlated with drastic frequency. High rainfall is therefore associated with high drastic frequency while low rainfall dictates low drastic frequency. The more favorable growing conditions found in areas of high rainfall influences food abundance. Areas with high rainfall, i.e., favorable environments, are accompanied by increased genetic diversity and, hence, increased drastic frequency. A negative correlation between temperature range and drastic frequency was also reported.

Band and Ives (1963) report an increase in lethal and semilethal mutation frequency following narrow temperature fluctuations as evidence for a dynamic relationship between the environment and the genetic load. Following an increase in temperature range conditions however, Band (1964) reports a significant decline in recessive drastic (lethal + semilethal) variants without overall lethal frequency decline. Such findings, she suggests, are due to differences in the effect of various environmental conditions on the prevailing genotype,

with natural selection acting primarily on heterozygotes. Apparently, selective advantage is at work and is experimentally realized by increased allelism within the prevailing population.

Modern industry is credited for introducing many known or suspected mutagenic or carcinogenic chemicals into the environment. The relationship between mutagenic and carcinogenic actions have been reviewed (Ames et al., 1975; Nagao and Sugimura, 1978). The induction of mutation is generally regarded as the initial first step in carcinogenesis (Purchase, 1982).

The importance of screening potential environmental mutagens and carcinogens became evident as epidemiological studies of differing populations suggested an environmental influence upon cancer incidence (Purchase, 1982). Such epidemiological analysis, and the traditional long-term animal test systems (i.e., heritable translocation, specific locus test, and dominant lethal assays in mice, among others), are being replaced in favor of cheaper, yet accurate, time-saving screening test systems.

Biological systems currently employed in sceening for potential mutagenic activity include microbial (Ames et al., 1975; Braun et al., 1977; Fumero and Mondino, 1977; Loprieno et al., 1979; Nagao and Sugimura, 1978; Purchase, 1982), mammalian cell culture (Nagao and

Sugimura, 1978; Loprieno et al., 1979; Purchase, 1982), and multicellular animals and plants (Lee et al., 1983).

Microbial test systems offer an economical and accurate means to assess mutagenic and carcinogenic risk. Two approaches in developing bacterial mutagenicity assays (a) back-mutation systems based on the reversion to prototrophy of auxotrophic strains and (b) forward-mutation systems based on inactivation of genes necessary for normal cell function, have been reported (Paes and Thompson, 1979). Of the bacterial test systems currently employed, the Salmonella typhimurium/mammalian microsome assay and the E. coli WP2 assay represent the most commonly employed, and most reliable systems (Purchase, 1982).

The in vitro transformation test using cultured mammalian cells provides an alternative to microbial screening for mutagenic or carcinogenic activity. Other mammalian test systems (i.e., forward mutation at the HGPRT locus in Chinese hamster cells, unscheduled DNA systhesis in EUE human cells, chromosomal aberration in mouse bone-marrow cells), are currently employed in mutagenic screening (Loprieno et al., 1979; Purchase, 1982). These tests generally require appropriate metabolic activation. Frequency of sister chromatid exchange (SCE) and cytotoxicity tests using fibroblasts from normal subjects and patients with xeroderma pigmentosum are also

employed in screening for mutagens and carcinogens. Although these tests are sensitive and specific, they are generally more costly than microbial test systems.

Mutagenic screening systems employing multicellular animals and plants are in widespread use. Although more costly than microbial or cell culture screening, inherent benefits include natural metabolic activation systems, defined genetic test protocol, high sensitivity and specificity. Purchase (1982) recommends multicellular animal test systems (i.e., Drosophila sex-linked recessive lethal [SLRL] assay, dominant lethal assays in mice, sperm abnormality in mice), in confirmatory testing of agents found mutagenic or carcinogenic in microbial systems. Fahrig (1977) recommends the mammalian spot test with mice for routine testing of environmental mutagens.

The present study utilizes D. melanogaster in examining potential genetic diversity of lethal frequencies between two natural populations. Population samples were obtained some three miles apart in Des Moines, Iowa; therefore, general geographic and weather variables are presumably quite similar. The first or control population was extracted from a wooded residential neighborhood in southwestern Des Moines, Iowa. The area is characterized by thick underbrush and tree growth surrounding a cultivated grass lawn. A ravine courses within

approximately 50 feet of this control locale, with runoff emptying into the Raccoon River.

The "experimental" or toxic population sample was extracted from an area adjacent to the Raccoon River near downtown Des Moines, Iowa. This site is similarly characterized by thick underbrush and trees, with an open grass lawn nearby. One variable reportedly associated with this site, however, is the presence of an underground plume of the degreasing solvent trichloroethylene or TCE, a suspected mutagen.

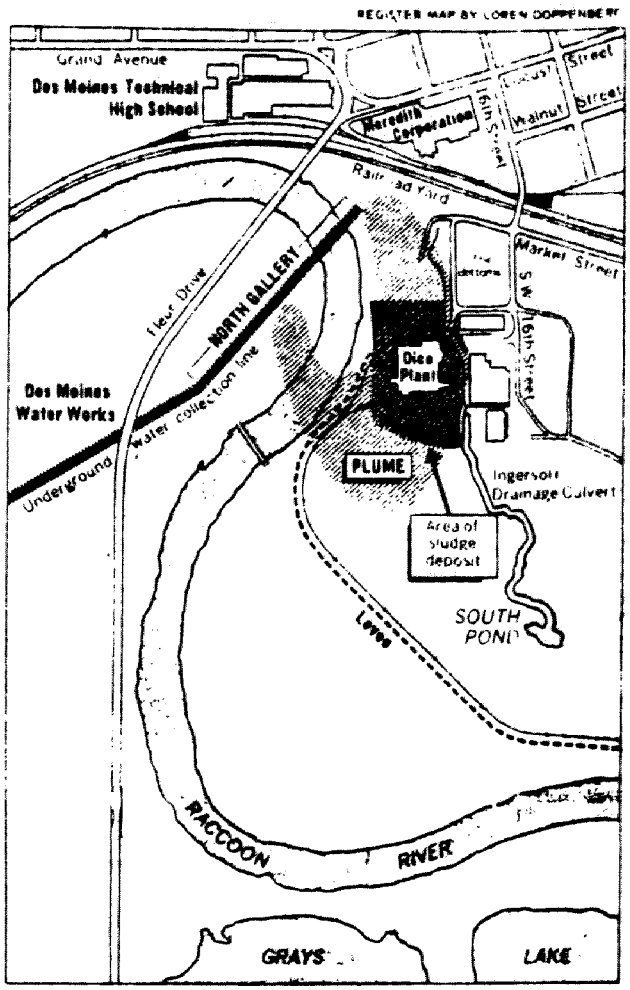
Trichloroethylene (1-chloro-2, 2-dichloroethylene; 1,1,2-trichloroethylene; trichloroethene; acetylene trichloride; trilene; TCE; $ClCH = CCl_2$) is widely used for vapor degreasing of fabricated metal parts, in dry cleaning, as a solvent, as an extractant in food processing, as a chain terminator for PVC production, as an anesthetic, a chemical intermediate, and as an ingredient in printing inks, paints, lacquers, varnishes, and adhesives (Fishbein, 1976, 1979).

Traces of TCE were initially detected in Des Moines area drinking water as early as 1974 (Santiago, 1985). Bullard (1985a) reports "The EPA advises against long-term consumption of water containing more than 75 parts per billion of TCE" (p. 2). He adds, "Two water samples collected in September 1983 contained 94 and 92 parts per billion and two samples taken a month later contained

65 and 60 parts per billion" (p. 2). Des Moines water has not exceeded 75 parts per billion of TCE since September 1983 (Bullard, 1985b); however, measures must be taken to reach the U.S. Environmental Protection Agency's proposed enforceable standard of five parts per billion (Bullard, 1985c).

Investigation to determine the source of the TCE contamination began in October 1984 (Norman, 1984). A report on the extent of the TCE plume and its proximity to the Des Moines Water Works' north gallery water collection line was recently completed by Ecology and Environment, Inc. (1985). This study included a gas chromatography-mass spectrometry (GC-MS) analysis of soils and groundwater samples from a 38-acre area. Figure 1 illustrates the toxic site (Santiago, 1985). A partial listing of metals and chemical compounds detected in this analysis is provided in Table 1.

Five potential sources of the TCE contamination were initially reported, including the Dico Co., Inc., at 200 S.E. Sixteenth Street, the closed Tuttle Street landfill two blocks east of the Dico plant, the former Solar Aircraft plant at Eighteenth Street and Grand Avenue (now housing Tech High School), the Meredith Corporation printing plant at 1716 Locust Street, and a dump just southeast of the Dico facility (Bullard, 1985a).



Map shows location of Dico plant and an underground plume of chemical TCE in relation to the Water Works' "north gallery" drinking water collection area.

Figure 1. Map of Des Moines Toxic Site*

* Map reproduced with permission of Des Moines Register (Santiago, 1985).

Table 1. Partial List of Chemicals Detected at Des Moines TCE Site

Aluminum	Aniline
Arsenic	Benzo-A-anthracene
Barium	Benzo-B-floranthene
Calcium	Bis (2-ethylhexyl) phthalate
Chromium	Butylbenzyl phthalate
Copper	Chloroform
Iron	Dianbutyl phthalate
Lead	Dianoctyl phthalate
Manganese	1,1-dichloroethane
Mercury	trans-dichloroethylene
Molybdenum	1,2-dichloropropane
Nickel	Endosulfan-1
Potassium	Fluoranthene
Sodium	Methylene chloride
Zinc	Nitrate
	4-nitrophenol
	Phenylchlorophenol
	Pyrene
	1,1,2,2-tetrachloroethene
	1,2,4-trichlorobenzene
	1,1,1-trichloroethane
	Trichloroethylene
	Vinyl chloride

The source of the TCE was determined to be the Dico Co., Inc. (Bullard, 1985c). Apparently officials at the facility ordered barrels full of waste sludge containing the chemical dumped onto their unpaved roads and parking lots in order to control dust in the early 1970s (Santiago, 1985). No report as to possible sources for the other contaminants has been found.

Recent experimental findings suggest TCE to be toxic, mutagenic, and carcinogenic. If consumed in large quantities, liver, kidney, and central nervous system damage may occur (Bullard 1985a). Greim et al. (1975) reports the finding that 1.0 mM of TCE resulted in 80-100% survival in E. coli strain K12.

"Trichloroethylene (TCE) is metabolized in vitro and in vivo to the scarcely reactive chloral hydrate and furtheron to trichloroethanol and trichloroacetic acid" (Greim et al., 1975, p. 2016). No known cytotoxic or genetic effects are associated with the latter two compounds.

Waskell (1978) tested TCE for direct interaction with PM2-DNA, finding no such interaction under the experimental conditions employed; however, chloral hydrate was found to exhibit weak mutagenic activity in the Ames Salmonella/rat-liver microsomal assay system.

TCE is reported to induce frameshift and base substitution mutation in Saccharomyces cereviscae strain

XV185-14C in the presence of mouse liver homogenate (Shahin and Von Borstel, 1977). It has also been shown to induce reverse mutation in E. coli strain K12 in the presence of a metabolic activating microsomal system (Fishbein, 1979).

Greim et al. (1975) report no mutagenic activity of the chlorinated ethylenes (including TCE), in E. coli strain K12 without metabolic activation; however, vinyl chloride ($ClCH = CH_2$), 1.1-dichloroethylene ($Cl_2C = CH_2$) and TCE were found to induce mutations in the gal^+, arg^+, MRT, and nad^+ systems.

Černá and Kypěnová (1977) report TCE-induced mutation to be dose-dependent in Salmonella typhimurium, strains TA1535 and TA1538, without metabolic activation. In a host-mediated assay of strains TA1950, TA1951 and TA1952 they also report increased reversion rates. Alternately, they report no significant mutagenic effect following cytogenetic analysis of mice bone-marrow cells after repeated intraperitoneal application. Similarly, Fishbein (1979) reports finding TCE mutagenic to S. typhimurium strains TA1535 and TA1538 without metabolic activation as well as finding increased reversion rates in strains TA1950, TA1951, and TA1952 in a host-mediated assay using female IRC mice.

Using the mammalian spot test, Fahrig (1977) treated embryos from the cross C57 BL/6J Han X T-stock in utero

by intraperitoneal injection of 1 mM TCE. Mutagenicity was indicated by brownish or light to dark grey color spots in otherwise black adult fur. White midventral spots were reported in response to pigment cell death, while nonwhite spots developed in response to gene mutation or recombination.

These findings are contradicted by Loprieno et al. (1979), who found TCE inactive in a mutagenicity multisystem test for: (1) reverse mutations to histidine independence in S. typhimurium; (2) forward mutations at 5 loci in S. pombe; (3) mitotic gene conversions at 2 loci in S. cerevisiae; (4) forward mutations at the HGPRT locus in V79 Chinese hamster cells; (5) unscheduled DNA synthesis in EUE human cells; and (6) chromosomal aberrations in mouse bone-marrow cells.

Trichloroethylene was found to be carcinogenic in B6C3F mice following gastric intubation (reported in Fishbein, 1976). Of the mice given the low dose (1200 mg/kg and 900 mg/kg for male and female respectively) 30.6% and of the mice given the higher dose (2400 mg/kg and 1800 mg/kg for male and female respectively) 43.2% developed hepatocellular carcinomas, with some metastases to the lungs; compared to a 2.5% carcinogenic development in control mice. No evidence regarding mutagenicity, or lack thereof, of trichloroethylene in Drosophila has been found.

Vinyl chloride (chloroethylene; ethylene monochloride; chloroethene; VCM; $CH_2 = CHCl$) is used in the production of homo-polymer (for PVC production) and co-polymer resins (e.g., Saran and other plastics), 1,1,1-trichloroethane (methyl chloroform), as a component of propellant mixtures and in specialty coatings (Fishbein, 1976, 1979).

Vinyl chloride undergoes biotransformation in mammals in a manner similar to that of TCE, with the first step being oxidation to oxirane (Greim et al., 1975), and has been demonstrated to be carcinogenic in animals and man (for references, see Greim et al., 1975). "The mutagenic action and other adverse biological effects of vinyl chloride appear to be dependent upon metabolic conversion into chemically reactive metabolites (e.g., chloroethylene oxide, 2-chloroacetaldehyde)" (Fishbein, 1976, p. 274). Greim et al. (1975) suggest the mutagenic activity of vinyl chloride to be the result of direct alkylating action of the oxirane. Mutagenicity of vinyl chloride has been demonstrated in (1) S. typhimurium; (2) E. coli strain K12; (3) S. pombe; and (4) Drosophila (for references, see Fishbein, 1976).

In order to evaluate a possible environmental influence on the genetic constitution in D. melanogaster, population samples extracted from the control and toxic locations previously described, were examined for

sex-linked (X) and autosomal (second chromosome) recessive lethal and semilethal genes. Frequency (proportion of lethal or semilethal genes detected) is thus used as an index of effect.

MATERIALS AND METHODS

A comparison of lethal and semilethal mutation frequencies in the first (X) and second chromosomes of two naturally occurring populations of Drosophila melanogaster was undertaken.

Collections

Flies were attracted to a fruit mash bait consisting of various aged fruits (especially bananas) and Bakers' yeast. Ten bait trays were randomly distributed within each collection site. Both the control and "toxic" sites have already been described. Bait trays consisted of paper plates containing two or three cups of fermented bait.

Regular collections (2-3/day) were made by sweeping an entomologist's net over the bait trays. All collections were made between October 7, 1985 and October 18, 1985. Rain and cold weather prevented further collections.

Upon collection, all flies were immediately transported to the laboratory in empty 25 x 105 mm glass

vials, where they were separated according to species and sex. Collection results for D. melanogaster are:

	♀♀	♂♂	Total
Toxic Site	72	45	117
Control Site	26	32	58

Stocks - Matings

Stock cultures for all research were maintained in 1/4 pint milk bottles, while experimental crosses were made in 25 x 105 mm. food vials. Food in all cases consisted of cornmeal, molasses, agar, and Brewers' yeast. Propionic acid was added to inhibit mold.

Although lighting varied within the laboratory, temperature remained relatively constant (22 ± 2°C) throughout the experiment.

All wild males collected, as well as single sons from each collected fertilized female were mated to virgin female stock of the genotype:

In(2L)Cy, Cy sp^2/In(2LR) bw^{vl}, ds^{33k} dp b bw^{vl};
In(3LR)DcxF, D/Sb.

Curly (Cy) and Plum (Pm) are dominant second chromosome markers. Dichaete (D) and Stubble (Sb) are dominant third chromosome markers. All four are lethal

when homozygous (Lindsley and Grell, 1967). Inversions associated with the second [i.e., In(2L); In(2LR)] and third [In(3LR)] chromosomes suppress crossing-over. Brown Variegated (bw^{vl}), a second chromosome marker, is recessive and lethal when homozygous (Lindsley and Grell, 1967). In the current analysis the fate of the second and third chromosome recessive gene markers is not relevant and is therefore disregarded in further discussions.

Examination for autosomal (second chromosome) recessive lethals was conducted by the Cy/Pm method (Dubinin, 1946; Wallace, 1950, 1956; Hiraizumi and Crow, 1960; Tobari, 1966).

Single virgin daughters of each fertilized female caught in each locality were anlyzed for sex-linked recessive lethals by the Basc or "Muller-5" method (Spencer and Stern, 1948; Muller et al., 1954; Lee et al., 1983; discussed for utility in Purchase, 1982).

The genotypic formula for the Basc test chromosome is:

$In(1)sc^{sIL}\ sc^{8R}\ In(1)S,\ sc^{8}\ sc^{sl}\ w^{a}\ B$

(Lindsley and Simm, 1979).

The Basc stock is constructed to facilitate the finding of sex-linked recessive lethals. It contains the dominant marker Bar (B) and the recessive apricot (w^{a}) as well as (to avoid the occurrence of

> crossover offspring) a long inversion-combination ($Insc^{sl}L$ - $Insc^{8}R$) associated with changes in the locus of scute (sc) and also a moderate-sized inversion (InS) included within the long one (Muller et al., 1954, p. 743).

The Basc stock is, furthermore, characterized by comparably high viability and fertility (Spencer and Stern, 1948).

Cy/Pm Test

The Cy/Pm genetic test is a method by which second chromosome homozygotes are obtained. Recessive lethals are detected in the F3 generation. The specific techniques involved are illustrated in Figure 2.

P1 x P1 matings were made using males collected from natural populations and virgin Cy/Pm female stock. Individual males were mated to three virgin females. The F1 progeny of interest are:

$$\frac{+^1}{Cy} \quad ; \quad \frac{+^2}{Cy} \quad .$$

($\pm^1$) and ($\pm^2$) represent alternate wild second chromosomes which may or may not contain lethal mutations. Subsequent testing will determine which condition is present. The F1 backcross enables isolation of each second chromosome in separate F2 cultures. The F2 progeny of interest are:

$$\frac{+^1}{Cy} \quad \text{and} \quad \frac{+^2}{Cy} \quad .$$

> crossover offspring) a long inversion-combination ($Insc^{sl}L$ - $Insc^{8}R$) associated with changes in the locus of scute (sc) and also a moderate-sized inversion (InS) included within the long one (Muller et al., 1954, p. 743).

The Basc stock is, furthermore, characterized by comparably high viability and fertility (Spencer and Stern, 1948).

Cy/Pm Test

The Cy/Pm genetic test is a method by which second chromosome homozygotes are obtained. Recessive lethals are detected in the F3 generation. The specific techniques involved are illustrated in Figure 2.

P1 x P1 matings were made using males collected from natural populations and virgin Cy/Pm female stock. Individual males were mated to three virgin females. The F1 progeny of interest are:

$$\frac{+^1}{Cy} \quad ; \quad \frac{+^2}{Cy} \quad .$$

($\pm^1$) and ($\pm^2$) represent alternate wild second chromosomes which may or may not contain lethal mutations. Subsequent testing will determine which condition is present. The F1 backcross enables isolation of each second chromosome in separate F2 cultures. The F2 progeny of interest are:

$$\frac{+^1}{Cy} \quad \text{and} \quad \frac{+^2}{Cy} \quad .$$

The F2 x F2 mating of sibs was made using three males and three females. This mating represents the actual genetic test in which identical homologs will be paired upon fertilization. As can be seen in Figure 2, there are two theoretical results obtained from this mating: (1) the wild second chromosome is normal or lacking lethal genes. This is represented by $(\frac{+}{+})$. In this instance, the F3 progeny will consist of two phenotypic classes, Curly and Wildtype, in a predictable ratio (2:1); (2) Should the wild second chromosome contain one or more lethal gene mutations the F3 progeny will all phenotypically show Curly wings.

Deviations from the theoretical or expected 2:1 ratio of Cy to Wildtype is ascribed to genes carried by the tested chromosome. Due to this variability, it becomes necessary to define precisely the method of classification. Following Tobari (1966), lethal chromosomes are defined as those chromosomes giving no Wildtypes when homozygous. Semilethals are chromosomes giving less than half the expected (33 1/3%) frequency of Wildtypes when homozygous, that is $(0\% < \frac{+}{+} < 16.7\%)$.

In order to verify positive lethal and semilethal F3 tests $(\frac{+}{+} < 16.7\%)$, an additional test generation was made, mating Curly F3 siblings. The F4 progeny ratios enabled confirmation or rejection of F3 results. This

was performed for all F3 lethal ($\frac{+}{+}$ = 0%) and many semilethal ($0 < \frac{+}{+} < 16.7\%$) F3 tests.

Basc Test

Single virgin daughters of each wild fertilized female captured were analyzed by the Basc or "Muller-5" method for the presence of X-linked recessive lethal genes. The mating scheme used is illustrated in Figure 3.

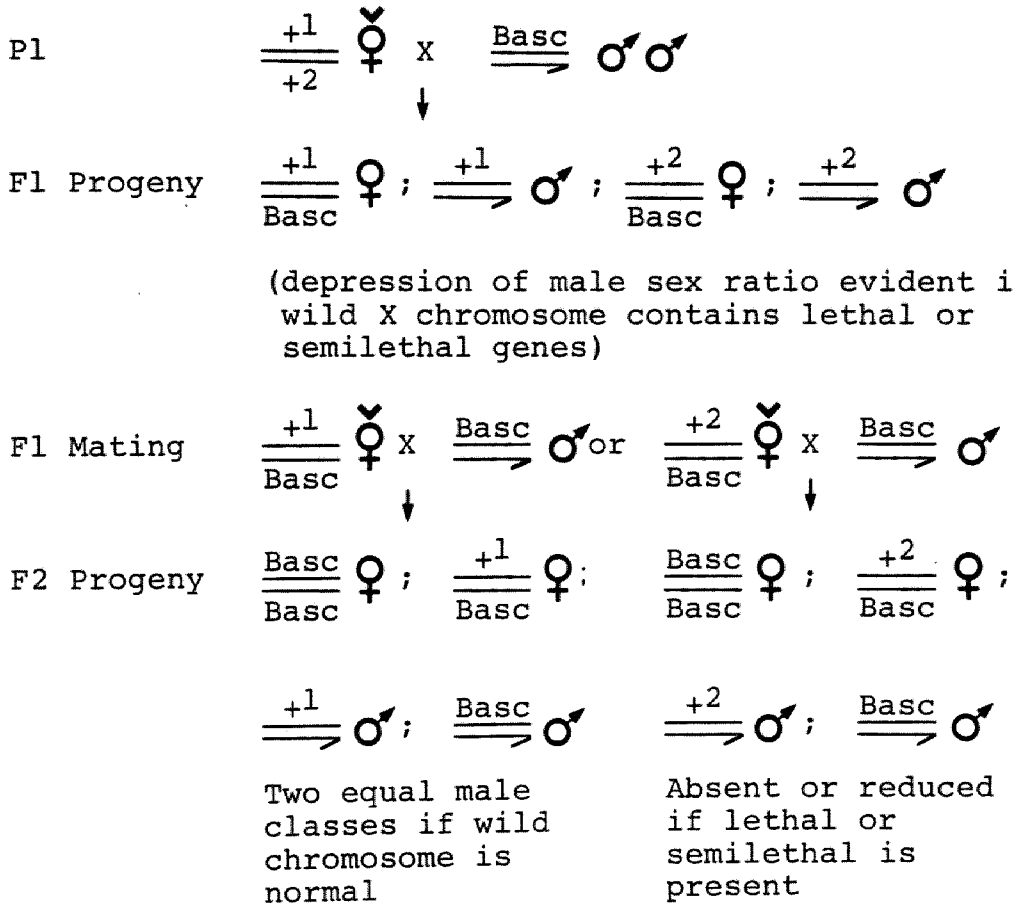


Figure 3. Basc Test for X-Linked Recessive Lethal Genes

Matings of P1 x P1 were made using Wildtype virgin females and Basc males. Individual females were mated to multiple males (3). The F1 female progeny thus obtained were Basc heterozygotes.

Seven F1 matings (virgin F1 Basc heterozygote females X Basc males) were prepared for each original P1 female. This measure allows high confidence [$(\frac{1}{2})^7$ = 99.2%] that both P1 X-chromosomes obtained from the wild ($\underline{+^1}$ and $\underline{+^2}$) would be examined. All F2 progeny were scored. As is evident from Figure 3, four phenotypic (and genotypic) F2 classes are expected unless the wild X-chromosome contains one or more recessive lethal gene mutations: Basc homozygote females; Basc heterozygote females; Basc males; Wildtype males. Should the "test" X-chromosome contain one or more recessive lethal genes ($+^2$ in Figure 3), the Wildtype male class will fail to appear. Alternately, if the wild "test" X-chromosome is normal (contains no lethal genes), Wildtype males will represent about 25% of the F2.

Presumptive positive tests (indicated by absence of F2 Wildtype males), were carried through an additional generation. In this mating, virgin F2 Basc heterozygote females were mated to Basc males. Examination of the F3 progeny allowed confirmation or rejection of the F2 test findings.

RESULTS

Table 2 presents the data for the Cy/Pm test. Among 312 fertile control F2 matings, 260 nonlethal, 30 semilethal, and 22 lethal presumptive tests were recorded. Following confirmation testing, 266 nonlethal, 26 semilethal, and 20 lethal control tests were recorded; resulting in 84.9% nonlethal, 8.7% semilethal, and 6.4% lethal control-test frequencies.

Among 492 fertile "toxic" F2 matings, 384 nonlethal, 50 semilethal, and 58 lethal presumptive tests were recorded. Following confirmatory testing, 405 nonlethal, 43 semilethal, and 44 lethal toxic tests were recorded, resulting in 82.3% nonlethal, 8.7% semilethal, and 8.9% lethal toxic-population test frequencies.

The control and toxic population samples are further classified according to their second chromosome constitution in Table 3. Classification of normal (n), lethal (le), and semilethal (sle) chromosomes was determined by the Cy/Pm method. Heterozygotic constitution was determined by an analysis of all data obtained in test generations F1 through F4.

The data in Table 3 show that wild flies containing no lethal or semilethal second chromosomes (n/n), in the control and toxic population samples analyzed represent 52% and 44% respectively. Viewed another way,

Table 2. Cy/Pm Test Data

		Control Population ♂♂	Toxic Population ♂♂
Total P1 Matings		45	95
Fertile P1 Matings		42	68
Total F1 Backcross Matings		336	537
Fertile F1 Backcross Matings		324	499
Total F2 Matings (Sibs)		324	499
Fertile F2 Matings		312	492
PRESUMED	Non-Lethal (F3)	260	384
	Semilethal (F3)	30	50
	Lethal (F3)	22	58
Total F3 Matings (Sibs)		36	82
Fertile F3 Matings		36	82
Total Non-Lethal		266	405
Total Semilethal (F4)		26	43
Total Lethal (F4)		20	44
TOTAL %	Non-Lethal	84.9	82.3
	Semilethal	8.7	8.7
	Lethal	6.4	8.9

48% of the control and 56% of the toxic population flies sampled in this study contained one or more drastic second chromosomes. The difference is not significant.

Table 3. Second Chromosome Constitution of Two Natural Drosophila populations.

	Second Chromosome Constitution								
	# n/n	(%)	# n/le	(%)	# n/sle	(%)	# le/sle	(%)	Total
Control Population	22	(52)	6	(14)	11	(26)	3	(7)	42
Toxic Population	30	(44)	18	(26)	15	(22)	5	(7)	68
Total	52	(47)	24	(22)	26	(24)	8	(7)	110

Data for the Basc test is presented in Table 4. The data show that only the toxic population sample contained X-linked recessive lethal genes in this study. The two presumptive F2 lethal tests were carried through an additional test generation (F3), as a check. This resulted in confirmation of one X-linked recessive lethal test and rejection of the other presumptive positive test.

Table 4. Basc Test Data

	Control Population ♀♀	Toxic Population ♀♀
Total P1 Matings	21	45
Fertile P1 Matings	19	34
Total F1 Matings	152	272
Fertile F1 Matings	138	238
% Fertile F1 Matings	91	88
Total Non-Lethal Tests (F2)	138	236
Total Presumptive Lethals and Number of F2 Matings	0	2
Total Lethal Tests Confirmed	0	1
% Lethal Tests	0	1.47

DISCUSSION

The present study examines the potential influence of environmental defilement on lethal gene mutation frequency in Drosophila melanogaster. The impetus for investigation originated from the chemical contamination of subsurface soil and waters near downtown Des Moines, Iowa.

Samples of wild D. melanogaster were captured within the reported toxic plume locale as well as a control area presumed to be free of chemical contamination. Lethal gene frequencies were determined for the first (X) and second chromosomes by the Basc and Cy/Pm methods, respectively. Both tests are well developed, hence, the utility of testing wild Drosophila populations for increased mutability, possibly resulting from environmental contamination, is evident (Dubinin, 1946; Purchase, 1982; Lee et al., 1983).

When the toxic population sample X-linked lethal mutation frequency of 1.47% is compared with the control population X-linked lethal mutation frequency of 0% the difference initially appears significant. Upon closer examination of the data, however, it becomes apparent that these figures were obtained from an analysis of small samples from each locality. This leads to the conclusion that the higher lethal frequency found in the X chromosomes of the toxic sample (the result of one

X-linked lethal), was likely due to chance rather than the result of increased genetic risk.

Results for the second chromosome drastic (lethal + semilethal) mutation frequencies may be viewed in two ways: (1) Drastic frequency of the overall fly population and (2) Drastic frequency of the chromosome population.

When the drastic frequency (of the overall fly population) within the toxic population sample (17.6%) is compared to the control population drastic frequency (15.1%), no significant difference is apparent. These results are consistent with those obtained by analyzing drastic gene frequencies in the respective chromosome populations. When the toxic population second chromosome drastic gene frequency of 56% is compared to the control population second chromosome drastic gene frequency of 48%, no significant difference exists. These figures are obtained by pooling n/le, n/sle, and le/sle data from Table 3 and rounding up the percentage figures. Alternately, similar figures may be obtained by subtracting the n/n percentage (representing flies containing only normal second chromosomes, see Table 3), from 100%.

Several possibilities exist regarding an interpretation of the data.

1. The chemical contamination of the toxic site is actually affecting the lethal mutation rate (and frequency) within the local Drosophila population, however,

the genetic analysis to establish a difference proved unsuccessful. Several factors could account for this occurrence: (a) The small samples analyzed were subject to sampling error and did not allow accurate lethal frequency calculation; (b) Late seasonal sampling, along with the unstable weather conditions encountered (both prior to and during sampling), altered the typical (and detected) lethal gene frequency. This could be explained by preferential selection for "normal" homozygotes. Hiraizumi and Crow (1960) found a 29% second chromosome drastic frequency for D. melanogaster, providing evidence in favor of this possibility. Testimony for seasonal fluctuation of drastic frequency has been presented (Band and Ives, 1963). Weather conditions would naturally have affected both the toxic and control populations somewhat equally, unless acting upon the chemicals in the environment or upon intermediates within the chemical-chromosomal interaction; (c) The affected chromosomes may have escaped analysis (i.e., the third and/or fourth chromosomes may have been acted upon preferentially by the chemicals contained in the toxic plume). This condition would seem unlikely; (d) The lethals, though present, may have escaped detection. This condition also seems unlikely due to the ramdom sampling employed during collection as well as the high specificity of the genetic tests employed.

2. The chemical contamination did not affect the lethal gene frequency within the toxic site population. Because the contamination largely involves subsurface soil and waters, it seems likely that flies within the area had little or no direct contact with the chemicals. Furthermore, because many of the chemicals concerned are highly volatile, any surface contamination would disperse rapidly. The foregoing suppositions would suggest the genetic analysis conducted to have been relatively accurate.

3. The control population sample lethal gene frequency may have been abnormally elevated, possibly the result of local environmental conditions (i.e., weather or chemicals). Because the toxic and control samples were obtained some three miles apart, differences related to weather are assumed to be negligible. Chemical analysis of control site soil and groundwater was not conducted, however it is assumed with high confidence that chemical contamination of the quality and quantity encountered in the toxic locale was absent.

If it is assumed that weather variables between the two sampling localities were equivalent and no differences related to food or water quality and availability were present, differences other than the chemical contamination of the toxic site are not obvious. This would lead to the conclusion that the chemical contamination concerned

in this study has no significant effect upon the lethal gene mutation frequency of the first (X) and second chromosomes in D. melanogaster under the conditions present in this study.

Conclusion

In conclusion, the present study found no significant relation between the reported chemical contamination of subsurface soil and waters near downtown Des Moines, Iowa and an increase in detectable X-linked and second chromosome lethal mutation frequency in D. melanogaster.

Further study to determine whether TCE presents a genetic threat to Drosophila is recommended as no such study has been found.

Use of the Basc and Cy/Pm test systems in monitoring environmental sites suspected or known to contain potential mutagenic or carcinogenic factors (i.e., chemicals, radiation), is suggested. Both tests are clearly defined and offer highly sensitive systems with objective scoring techniques and specific results capable of detecting mutagenic action.

LITERATURE CITED

Altenburg, E. A quantitative study of mutation in the X-chromosome of Drosophila. Unpublished paper; 1919.

Ames, B. N.; McCann, J.; Yamasaki, E. Methods for selecting carcinogens and mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test. Mutation Research 31: 347-364; 1975.

Band, H. T. Genetic structure of populations. III. Natural selection and concealed genetic variability in a natural population of Drosophila melanogaster. Evolution 18:384-404; 1964.

Band, H. T.; Ives, P. T. Genetic structure of populations. I. On the nature of the genetic load in the South Amherst population of Drosophila melanogaster. Evolution 17:198-215; 1963.

Band, H. T.; Ives, P. T. Genetic structure of populations. IV. Summer environmental variables and lethal and semilethal frequencies in a natural population of Drosophila melanogaster. Evolution 22:633-641; 1968.

Braun, R.; Zander, M.; Adler, B. Comparison of DNA damaging activity of chemical mutagens and carcinogens in bacteria with different DNA repair capacities. Mutation Research 46:214; 1977.

Bullard, C. Tainted water in D.M. still puzzles EPA. Des Moines Register. 1985a Feb. 28; Sect. M:1.

Bullard, C. Water Works officials accused of inaction on TCE contamination. Des Moines Register. 1985b May 21; Sect. A:1, 8.

Bullard, C. Experts at odds on source of chemical in D.M. water. Des Moines Register. 1985c July 13; Sect. A: 1, 16.

Černá, M.; Kypěnová, H. Mutagenic activity of chloroethylenes analysed by screening system tests. Mutation Research 46:214-215; 1977.

Demerec, M. Frequency of spontaneous mutations in certain stocks of Drosophila melanogaster. Genetics 22: 469-478; 1937.

Dobzhansky, Th. Experimental studies on genetics of free-living populations of Drosophila. Biol. Rev. 14:339-368; 1939.

Dobzhansky, Th.; Queal, M. L. Genetics of natural populations. II. Genic variation in populations of Drosophila pseudoobscura inhabiting isolated mountain ranges. Genetics 23:463-484; 1938.

Dobzhansky, Th.; Spassky, B. Genetics of natural populations. XI. Manifestation of genic variations in Drosophila pseudoobscura in different environments. Genetics 29:270-290; 1944.

Dobzhansky, Th.; Spassky, B. Genetics of natural populations. XXI. Concealed variability in two sympatric species of Drosophila. Genetics 38:471-484; 1953.

Dobzhansky, Th.; Wright, S. Genetics of natural populations. V. Relations between mutation rate and accumulation of lethals in populations of Drosophila pseudoobscura. Genetics 26:23-51; 1941.

Dubinin, N. P. On lethal mutations in natural populations. Genetics 31:21-38; 1946.

Ecology and Environment, Inc. Final remedial investigative report, Des Moines TCE site. CH_2M Hill, Inc., Kansas City, Missouri, Vol. 1-4; Dec. 1985.

Fahrig, R. The sensitivity of the mammalian spot test to mutagens of different types of action. Mutation Research 46:202; 1977.

Fishbein, L. Industrial mutagens and potential mutagens. I. Halogenated aliphatic derivatives. Mutation Research 32:267-308; 1976.

Fishbein, L. Potential industrial carcinogens and mutagens. Amsterdam, Netherlands: Elsevier Sci. Publ. Co., 1979, pp. 182-187.

Fumero, S.; Mondino, A. Mutagenicity of industrial compounds evaluated by means of yeast genetic systems. Mutation Research 46:189; 1977.

Greim, H.; Bonse, G.; Radwan, Z.; Reichert, D.; Henschler, D. Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation. Biochemical Pharmacology 24:2013-2017; 1975.

Herskowitz, I. H. A list of chemical substances studied for effects on Drosophila, with a bibliography. The American Naturalist 85(822):181-199; 1951.

Hiraizumi, Y.; Crow, J. F. Heterozygous effects on viability, fertility, rate of development and longevity of Drosophila chromosomes that are lethal when homozygous. Genetics 45:1071-1083; 1960.

Ives, P. T. Heterogeneity in the lethal mutation rate within a wild population of Drosophila melanogaster (abst.). Genetics 28:77; 1943.

Ives, P. T. The genetic structure of American populations of Drosophila melanogaster. Genetics 30:167; 1945.

Lee, W. R.; Abrahamson, S.; Valencia, R.; vonHalle, E. S.; Wurgler, F. E.; Zimmering, S. The sex-linked recessive lethal test for mutagenesis in Drosophila melanogaster. A report of the U.S. Environmental Protection Agency gene-tox program. Mutation Research 123:183-279; 1983.

Lefevre, G. The distribution of randomly recovered x-ray induced sex-linked genetic effects in Drosophila melanogaster. Genetics 99:461-480; 1981.

Lindsley, D. L.; Grell, E. H. Genetic variations of Drosophila melanogaster. Oak Ridge, Tennessee: Carnegie Institution of Washington Publications; 1967.

Lindsley, D. L.; Simm, G. G. Computer stock list. Drosophila Information Service 54:vi; 1979.

Loprieno, N.; Barale, R.; Presciuttini, S.; Rossi, A. M.; Sbrana, I.; Stretti, G.; Zaccaro, L.; Abbondandolo, A.; Bonatti, S.; Fiorio, R. Comparative data with different test systems using micro-organisms and mammalian cells on references and environmental mutagens. Mutation Research 64:119; 1979.

Muller, H. J. Artificial transmutation of the gene. Science 66:84-87; 1927.

Muller, H. J. The measurement of gene mutation rate in Drosophila, its high variability and its dependence upon temperature. Genetics 13:279-357; 1928.

Muller, H. J.; Altenburg, E. The rate of change of hereditary factors in Drosophila. Proc. Soc. Exp. Biol. Med. 17:10-14; 1919.

Muller, H. J.; Herskowitz, I. H.; Abrahamson, S.; Oster, I. I. A nonlinear relation between x-ray dose and recovered lethal mutations in Drosophila. Genetics 39:741-749; 1954.

Nagao, M.; Sugimura, T. Environmental mutagens and carcinogens. Ann. Rev. Genet. 12:117-149; 1978.

Norman, J. EPA sleuths to seek TCE origin in DM. The Des Moines Register. 1984 Oct. 5; Sect. M:1, 2.

Olenov, J. M.; Kharmac, I. S.; Galkovskaya, K. F.; Muretov, G. D. Factors responsible for the genic composition of wild Drosophila melanogaster populations. C. R. (Doklady) Acad. Sci. URSS 24:466-470; 1939.

Paes, D.; Thompson, S. Forward mutagenesis as a test system in Salmonella typhimurium. Mutation Research 64:119-120; 1979.

Purchase, I. F. H. An appraisal of predictive tests for carcinogenicity. Mutation Research 99:53-71; 1982.

Santiago, F. Chemicals used to settle dirt bring unsettling results. Des Moines Register. 1985 May 19: Sect. A:1, 12.

Shahin, M. M.; Von Borstel, R. C. Mutagenic and lethal effects of ∝- benzene hexachloride, dibutyl phthalate and trichloroethylene in Saccharomyces cerevisiae. Mutation Research 48:173-180; 1977.

Spencer, W. P.; Stern, C. Experiments to test the validity of the linear R-dose/mutation frequency relation in Drosophila at low dosage. Genetics 33: 43-74; 1948.

Stadler, L. J. Mutations in barley induced by x-rays and radium. Science 68:186-187; 1928.

Tobari, I. Effects of temperature on the viabilities of homozygotes and heterozygotes for second chromosomes of Drosophila melanogaster. Genetics 54:783-791; 1966.

Wallace, B. Autosomal lethals in experimental populations of Drosophila melanogaster. Evolution 4:172-174; 1950.

Wallace, B. Studies on irradiated populations of Drosophila melanogaster. J. Genet. 54:280-292; 1956.

Waskell, L. A study of the mutagenicity of anesthetics and their metabolities. Mutation Research 57:141-153; 1978.

Watson, J. D.; Crick, F. H. C. Genetical implications of the structure of deoxyribonucleic acid. Nature 171: 964-967; 1953.